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Third Director of the European Centre for Disease Prevention and Control takes office

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On Friday 16 June 2017, Dr Andrea Ammon took up office as the third Director of the European Centre for Disease Prevention and Control (ECDC) following her election by the Centre's management board earlier the same year. The appointment follows a two-year tenure as acting director during which Dr Ammon steered the ECDC steadily and calmly through a challenging period when besides the Centre's day-to-day work, expertise and resources were requested for the European preparedness and response to global threats such as the Ebola and Zika virus disease outbreaks in Africa and the Americas [1,2].

Dr Ammon joined the newly established ECDC already in May 2005, as one of its first employees and Head of the Surveillance Unit [3]. While still setting up the unit, she was instrumental in drafting and implementing a long-term surveillance strategy for the European Union (EU). As part of this, she and her enthusiastic team evaluated the existing 17 European Dedicated Surveillance Networks (DSN) which included well-established and widely known networks such as EURO TB and EURO HIV, and gradually transferred them into the ECDC [4]. In parallel, her unit developed The European Surveillance System (TESSy), revised the EU case definitions and produced for the first time an Annual Epidemiological Report on infectious diseases in the EU.

From April 2011 to April 2015, Andrea Ammon was Deputy to the Director and ECDC's Head of Unit for Resource Management and Coordination. She was a member of the scientific committee for the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) and in 2014 and 2015, she headed the committee.

A medical doctor by training, Dr Ammon discovered her passion for public health early in her career and she has extensive experience in working in public health authorities at differing levels. Starting at the local and then regional level in the German federal state of Bavaria, she moved to the national public health institute, the Robert Koch Institute (RKI) in 1996, where she was among the first national Field Epidemiology Programme trainees and simultaneously a member of the first cohort of the European Programme for Intervention Epidemiology Training (EPIET). At RKI, she became the Head of Department for Infectious Disease Epidemiology and State Epidemiologist for Germany from late 2002 to 2005. Besides coordinating the national outbreak response team for current and emerging infections, she directed the national field epidemiology training programme and coordinated emergency planning for influenza and epidemiological research programmes in infectious diseases. Furthermore, she provided scientific advice for government ministries, Members of Parliament and the public. In 2003, she coordinated the German response to Europe's first imported case of severe acute respiratory syndrome (SARS). During her time at RKI, Dr Ammon also became a nationally and internationally respected expert in the field of food- and waterborne diseases. Her PhD was on the synergy between epidemiology and microbiology in the prevention and control of food-borne diseases.

Dr Ammon's professional and leadership skills are complemented by other strong characteristics such as a mind open to suggestions, a capacity for motivating staff and an acute sense of fairness.

The *Eurosurveillance* journal and its editors benefited from Dr Ammon's strategic vision and sense for quality between 2007 and 2015, when she was an associate editor and a strong supporter of the journal. She resigned from this position when taking up her post as ECDC Director to mark the editorial independence of the journal from its publisher and its Director.

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Fall in new HIV diagnoses among men who have sex with men (MSM) at selected London sexual health clinics since early 2015: testing or treatment or pre-exposure prophylaxis (PrEP)?

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Since October 2015 up to September 2016, HIV diagnoses fell by 32% compared with October 2014–September 2015 among men who have sex with men (MSM) attending selected London sexual health clinics. This coincided with high HIV testing volumes and rapid initiation of treatment on diagnosis. The fall was most apparent in new HIV testers. Intensified testing of high-risk populations, combined with immediately received anti-retroviral therapy and a pre-exposure prophylaxis (PrEP) programme, may make elimination of HIV achievable.

Gay, bisexual and other men who have sex with men (MSM) account for half of all people living with HIV in England and are the group most at risk of acquiring HIV [1]. By end 2015, 94% (34,439/37,590) of MSM diagnosed with HIV in England received anti-retroviral therapy (ART), of whom 95% had supressed viral load (viral load < 200 mL) [1]. An additional 5,000–8,000 MSM were estimated to have undiagnosed infection [1-3].

Since 2012, national guidelines have recommended up to 3-monthly HIV testing for MSM at high risk of acquiring HIV [4,5] and starting ART regardless of CD4 count to prevent onward transmission ('treatment as prevention') [6,7]. Consequently, the number of men starting ART rose from 2,700 in 2013 to 3,600 in 2015 [1]. Beginning in 2013, pre-exposure prophylaxis (PrEP) has been available to some MSM as part of the 'Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD)' trial [8] and more recently through international purchasing online [9,10]. In December 2016, selected London sexual health clinics reported a fall in HIV diagnoses among MSM [11]. A rapid analysis of surveillance and monitoring data was conducted to confirm and explain this fall.

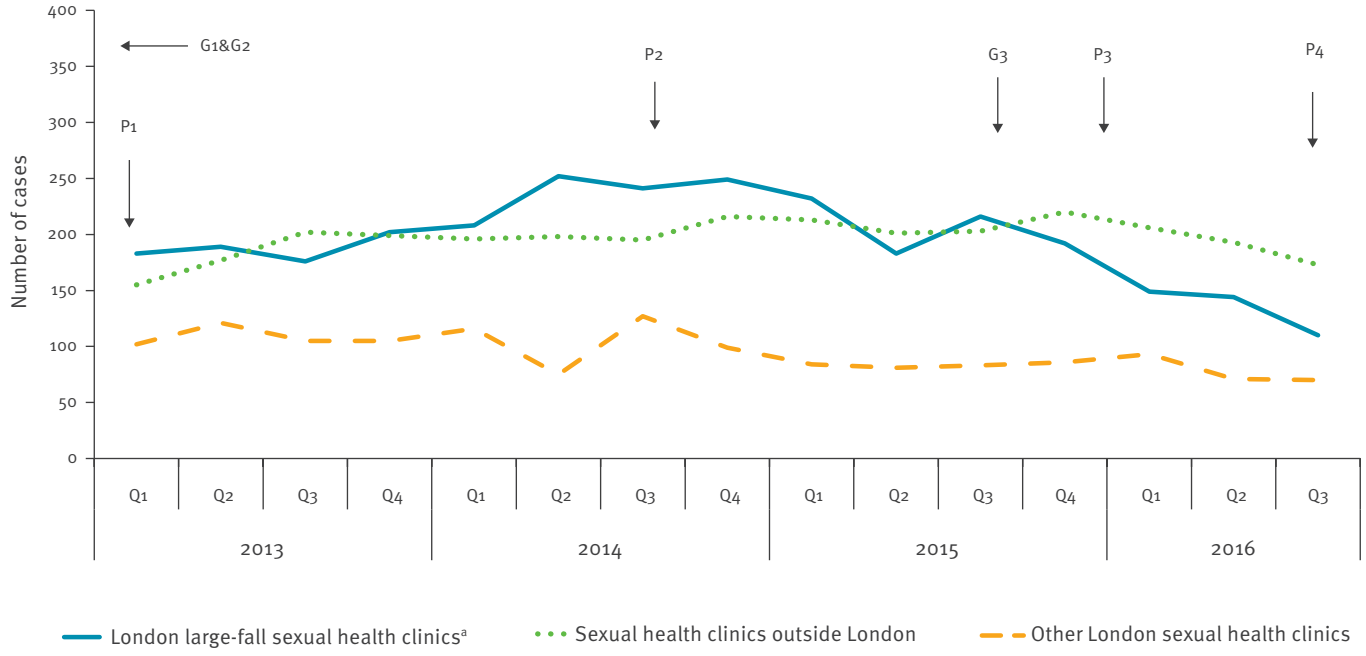
Data sources and analysis

Quarterly data from the genitourinary medicine clinic activity dataset (GUMCADv2) for January 2013–September 2016 [12] were used to examine HIV diagnoses and testing patterns among MSM attending one of the over 200 free, confidential, open-access sexual health clinics in England. Clinics that reported a large fall in diagnoses in the most recent year for which data were available, i.e. clinics with a > 20% decline and > 40 cumulative new HIV diagnoses between October 2014–September 2015 and October 2015–September 2016, were compared with other clinics in London and outside London. The number of HIV-negative MSM attending with a history of an HIV test and a bacterial sexually transmitted infection (STI) (> 90% were genital or rectal infections) was used as an indicator for those at high risk of HIV acquisition.

The HIV and AIDS Reporting System (HARS) [13] data, geographically aligned for clinics, for the most recent years (2013–2015) were used to examine: (i) trends in CD4 count within 91 days of HIV diagnosis; (ii) the number of MSM diagnosed with HIV who are untreated or treated but whose viral load is not suppressed; and (iii) time from HIV diagnosis to ART initiation. National estimates of HIV prevalence were stratified by the proportion of diagnosed and undiagnosed infection [3]. Estimates of the proportion of MSM with undiagnosed HIV infection [1] were calculated using the number of MSM with diagnosed infection to estimate the number of those undiagnosed in the catchment area of each clinic group.

Figure 1

New HIV diagnoses among men who have sex with men attending sexual health clinics by year and quarter, England, 2013–2016 (n = 7,291 HIV diagnoses)



BASHH: British Association for Sexual Health and HIV; BHIVA: British HIV Association; MSM: men who have sex with men; PrEP: pre-exposure prophylaxis; PROUD: Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection; Q = quarter.

[a] Defined as clinics with at least a 20% decrease in HIV diagnoses and over 40 cumulative diagnoses between October 2014–September 2015 and October 2015–September 2016.

P1: Recruitment to the PROUD trial began with 50% of participants allocated to the deferred arm (12 months after trial commenced) [8]. P2: All deferred participants offered PrEP. P3: Online purchasing of PrEP began in late 2015. P4: Online purchasing of PrEP routine. Interpolating from the trial enrolment figures over time [5], an estimated 200 MSM were taking PrEP by end 2013, 500 by end 2014, and it is likely an additional few hundred by end 2016 with the majority of the latter via online purchase [9,10,14,15].

G1: In May 2012 BHIVA/ BASHH and in November 2012, Public Health England (at the time, the Health Protection Agency) recommended that high-risk MSM have an HIV test annually and up to every 3 months if having condomless sex with new or casual partners.

G2: In May 2012, BHIVA guidelines advised people could start ART with a CD4 count › 350 cells after discussion with the clinician should the patient wish to protect partners from sexual HIV transmission.

G3: In September 2015, BHIVA guidelines were strengthened to recommend treatment for all individuals regardless of CD4 count for the purpose of treatment as prevention.

Fall in HIV diagnoses among men who have sex with men

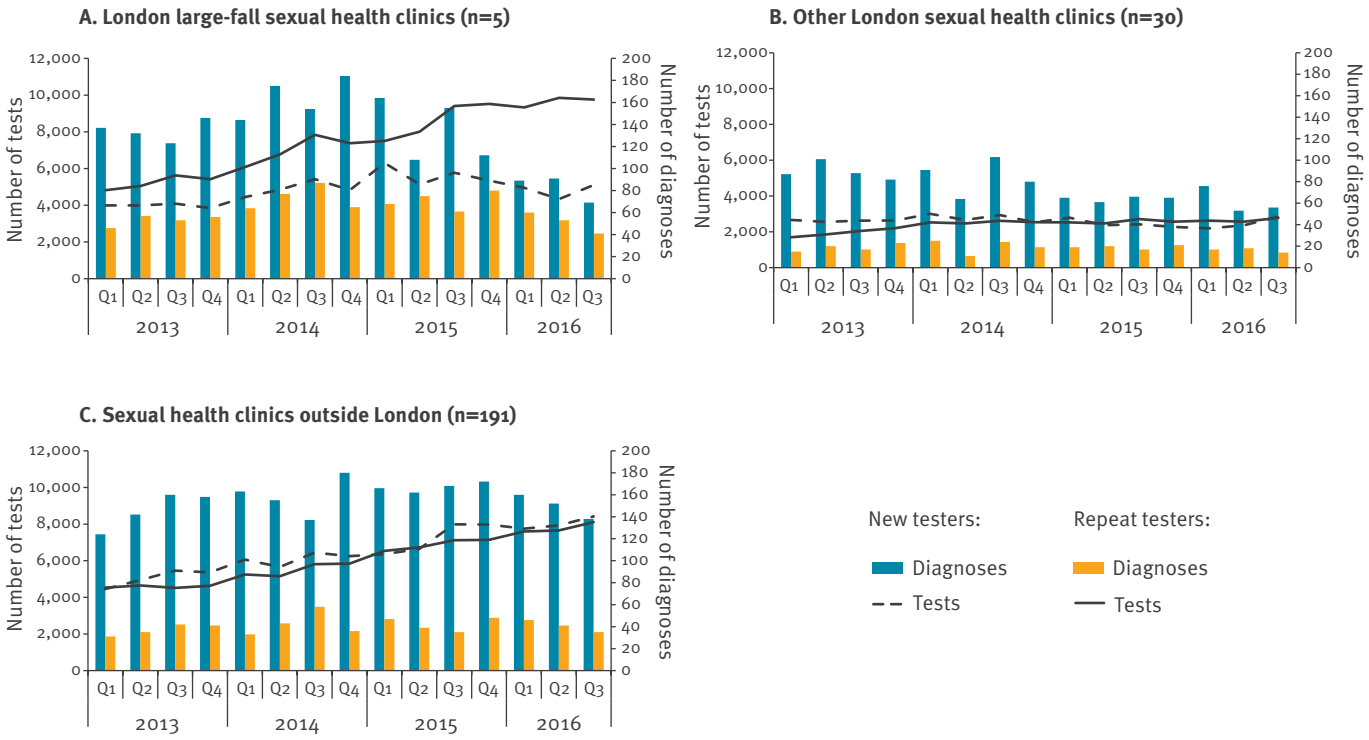
Between October 2014–September 2015 and October 2015–September 2016, reported new HIV diagnoses among MSM fell by 17% (from 2,060 to 1,707) in England and by 25% (from 1,227 to 915) in London. Nationally, diagnoses among heterosexuals remained stable at 1,500 in both periods. A 32% decline was observed among five London large-fall clinics (from 880 to 595; p = 0.014 for test of linear trend in diagnoses by quarter) compared with 8% at 30 other London clinics (from 347 to 320, p = 0.115) and 5% (from 833 to 792, p = 0.101) in 191 clinics in the rest of England (Figure 1,2).

Changing patterns of HIV testing

Testing patterns were analysed from January 2013–September 2016. Among the large-fall clinics (Figure 2a), the number of HIV tests in MSM increased by 50% (from 8,820 in January–March 2013 to 14,820 in July–September 2016); the number of new testers, i.e. those not tested in the previous 2 years, was stable at around 5,000 per quarter, whereas the number of repeat testers i.e. those who had an HIV test within the previous 2 years increased by 60%, from 4,800 to 9,760. The 3-year rise in testing in the large-fall clinics coincided with an initial increase in HIV diagnoses through 2014 in both new and repeat testers and in early 2015 the decline was observed, predominantly in new testers. In other London clinics, the number of new and repeat

Figure 2

Number of HIV tests and diagnoses in men who have sex with men at sexual health clinics by new and repeat tests and clinic group, England, 2013–2016



Q: quarter.

testers remained stable, and outside London, new and repeat testers increased equally, although there was no discernible effect on HIV diagnoses in either setting (Figure 2b and c).

Over the period, the number of MSM attending clinics increased by 4% for both groups of London clinics, and by 16% outside of London. Importantly, the volume of testing at the large-fall clinics was such that 41% (58,180/140,980) of HIV tests in MSM attending clinics in England during October 2015–September 2016 occurred at one of these five clinics. Exceptionally, the median CD4 count at HIV diagnosis of men diagnosed at large-fall clinics increased substantially (from 469 in 2013 to 548 in 2015). In contrast, the median CD4 count rose only from 442 to 489 in other London clinics, and remained around 430 outside London, over the same period. This indicates that the testing volumes and frequency of testing carried out in these settings were still insufficient to substantially reduce the average time from infection to diagnosis compared with large-fall clinics.

Prompt treatment following HIV diagnosis

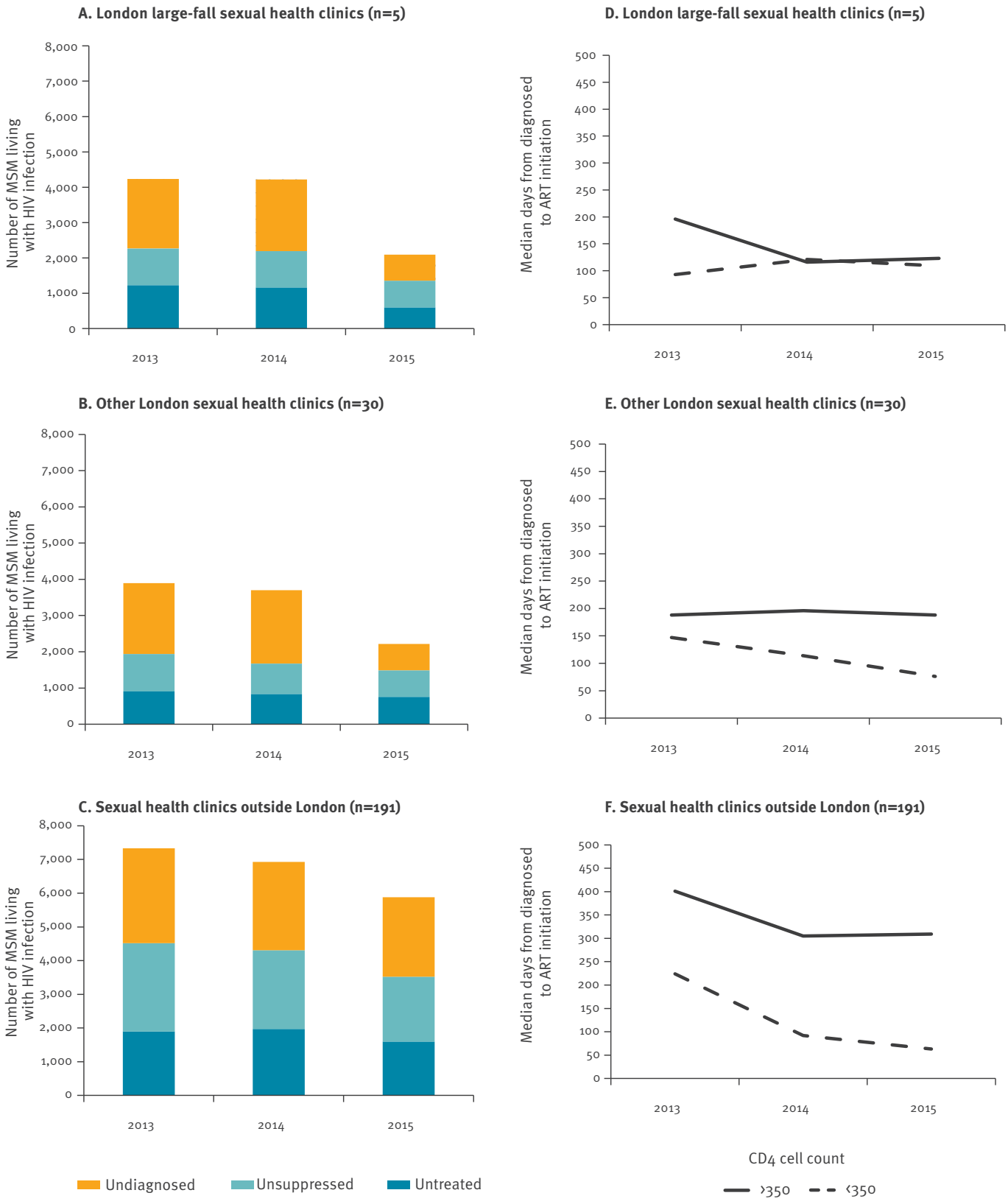
Although the number of MSM living with diagnosed HIV infection who were untreated declined by 27% in England (from 4,025 in 2013 to 2,950 in 2015), this decline was greatest at large-fall clinics (51%; from 1,224 to 601) compared with other London clinics (17%; from 906 to 754) and clinics outside London (16%; from 1,895 to 1,595) (Figures 3a-c). Moreover, while there has been a general reduction in the time to starting ART in those with a CD4 count›350 at onset of ART, the median time from diagnosis to treatment in 2015 was substantially shorter at large-fall clinics (120 days) compared with other London clinics (190 days) and clinics outside London (260 days) (Figures 3d-f).

Men who have sex with men with transmissible levels of virus

MSM with transmissible levels of virus include those diagnosed who are untreated or treated with a viral load›200 copies/mL, as well as those with an undiagnosed infection (Figure 3a-c). In 2015, there were an estimated 10,190 MSM with transmissible levels of virus in England: 29% (n=2,950) untreated, 34% (n=3,420) unsuppressed and 37% (n=3,820) undiagnosed. In the same year, among clinic attendees, the ratio of MSM with transmissible levels of virus to MSM at high-risk of HIV acquisition was 0.6 (2,088/3,596) at large-fall clinics, 2.6 (2,219/868) at other London clinics and 2 (5,877/2,933) at clinics outside London. Assuming that sexual networks broadly correspond with clinic attendance patterns, the documented ratio differences suggest that MSM at high risk of HIV acquisition who attended one of the large-fall clinics have a

Figure 3

Numbers of men who have sex with men living with HIV infection who are undiagnosed, diagnosed and untreated or treated and non-supressed viral load (A-C) and median time (days) from HIV diagnosis to ART initiation, by CD4 count at ART start (D-F) by clinic group, England, 2013–2015



ART: anti-retroviral therapy; MSM: men who have sex with men.

Treatment data and viral load data are adjusted for missing information (99% and 84% respectively): CD4 cell count taken within 91 days of diagnosis, available for 91% (7,519/8,297) of records; CD4 count at ART initiation, available for 76% (6,960/9,150) of records. Year-specific estimates of proportion of all MSM with HIV who are undiagnosed were obtained using Multi-Parameter Evidence Synthesis [1,3].

much lower likelihood of exposure to a man with transmissible levels of virus.

Availability of pre-exposure prophylaxis

Available data suggest the number of MSM who began PrEP in England either as trial participants or via online purchase has been limited to date. Although all five large-fall clinics participated in the PROUD trial, three other clinics in London and five clinics outside London did so as well. An estimated 200 MSM were taking PrEP by end 2013, 500 by end 2014 [8,14] and it is likely an additional few hundred by end 2016 [9,10,15].

Assuming a best prevention case scenario of a 9% annual HIV incidence, the very high-risk level as observed in the PROUD trial [8], by end 2015, the cumulative number of HIV infections directly prevented by PrEP would have been 90 at most. Not all of them would have attended large-fall clinics and of those who did, the decline in directly prevented infections would have been most apparent in repeat HIV testers.

Limitations

Though powerful, the surveillance and monitoring data needs cautious interpretation, especially given the post-hoc nature of the analysis. Conclusions could be affected by reporting delay (albeit minimal), incomplete data in relation to ART coverage, ART start date and CD4 count at HIV diagnosis, and neither the impact of partner notification nor the movement between clinics for HIV testing is measured. The assumption that attendees of the same clinics are more likely to form part of the same sexual network compared with random sexual mixing is plausible but unsubstantiated. Finally, while numbers of HIV diagnoses are not synonymous with HIV incidence, the rise in median CD4 count at HIV diagnosis suggests that the fall in diagnoses reflects a fall in incidence.

Conclusions

The 17% fall in new HIV diagnoses in MSM in England between October 2014–September 2015 and October 2015–September 2016 was focussed in five clinics which experienced a 32% decline. The fall seen at these five clinics coincided with accelerated treatment at diagnosis and a substantial increase in HIV testing, particularly repeat testing.

The volume of HIV tests across London combined with rapid treatment following diagnosis at the five large-fall clinics is now likely to have reached a level that decreases the number of men with transmissible levels of virus thereby reducing transmission. The use of PrEP among high-risk MSM, although limited at this stage, will also have contributed to the fall in new diagnoses. If HIV testing of MSM at high risk of HIV is intensified, and wide-scale immediate ART, as observed within the London large-fall clinics, is replicated elsewhere, it is probable that a substantial reduction in HIV transmission among MSM could be achieved nationally. Should the promise of the 'PrEP Impact Trial' proposed in England [16] be realised promptly, then a very large reduction in HIV transmission in MSM may be attained. The similarity of the MSM HIV epidemic in England to elsewhere in western Europe [17] suggests a similar approach in these countries might be equally successful.

Conflict of interest

None declared.

Authors' contributions

The analysis was performed by: AB, HM, DO, PK, MY, SN, MF. AB, HM, NC, GH, VD and ON were responsible for conceiving, developing and interpreting the analyses. AB and HM led the writing jointly with significant input from VD and ON.

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Genomic investigation of a suspected outbreak of *Legionella pneumophila* ST82 reveals undetected heterogeneity by the present gold-standard methods, Denmark, July to November 2014

S Schjørring [1,2], M Stegger [1], C Kjelsø [3], B Lilje [1], JM Bangsborg [4], RF Petersen [5], S David [6], SA Uldum [1], on behalf of the ESCMID Study Group for Legionella Infections (ESGLI)

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Between July and November 2014, 15 community-acquired cases of Legionnaires´ disease (LD), including four with *Legionella pneumophila* serogroup 1 sequence type (ST) 82, were diagnosed in Northern Zealand, Denmark. An outbreak was suspected. No ST82 isolates were found in environmental samples and no external source was established. Four putative-outbreak ST82 isolates were retrospectively subjected to whole genome sequencing (WGS) followed by phylogenetic analyses with epidemiologically unrelated ST82 sequences. The four putative-outbreak ST82 sequences fell into two clades, the two clades were separated by ca 1,700 single nt polymorphisms (SNP) s when recombination regions were included but only by 12 to 21 SNPs when these were removed. A single putative-outbreak ST82 isolate sequence segregated in the first clade. The other three clustered in the second clade, where all included sequences had < 5 SNP differences between them. Intriguingly, this clade also comprised epidemiologically unrelated isolate sequences from the UK and Denmark dating back as early as 2011. The study confirms that recombination plays a major role in *L. pneumophila* evolution. On the other hand, strains belonging to the same ST can have only few SNP differences despite being sampled over both large timespans and geographic distances. These are two important factors to consider in outbreak investigations.

Introduction

Legionnaires' disease (LD) is notifiable in Denmark. When *Legionella* isolates are obtained these can be voluntarily submitted to the Statens Serum Institut (SSI) for identification and typing. The surveillance system for LD combines information from the notifications with any respective available typing data. In the summer/autumn of 2014, Denmark observed an increase in LD cases compared with previous years [1]. In the North Zealand region, between July and November, 15 cases (75 cases/1.000.000/year) were notified. Comparatively, in the same period of the four previous years (2010 to 2013), an average of 5.5 (range 1 – 10) community-acquired LD cases were diagnosed (equivalent to 27.5 cases/1.000.000/year) in the region.

Among the 15 LD cases related to North Zealand in 2014, four were infected with *L. pneumophila* serogroup 1 subgroup Allentown/France, sequence type (ST) 82. ST82 has been only observed in six cases of LD since 2009 in different parts of Denmark but all outside the North Zealand region, and never in environmental samples. Three of the historical cases were associated with travel or were of unknown origin and three were community-acquired cases with no epidemiological links. Thus, the detection of four cases with ST82 within five weeks and an overall high incidence of LD in the region led to a hypothesis of a LD outbreak with a common environmental source.

In this study, we used standard epidemiological and typing tools as well as whole genome sequencing (WGS) to retrospectively investigate the putative LD outbreak.

Methods

Case definitions and data source

The European Union (EU) case definition for confirmed and probable cases of LD [2] was used. All cases in this study had pneumonia. Confirmed cases were diagnosed by culture and/or urinary antigen tests and probable cases were diagnosed by PCR only. The outbreak case definition was a person with LD diagnosed at the Department of Clinical Microbiology (DCM) of the regional hospital between 30 June and 19 November 2014 with no hospitalisation or travel during the incubation period (4 to 10 days before onset of symptoms). Information on confirmed/probable LD cases was extracted from the surveillance database at SSI and from the records of the DCM at the regional hospital.

Epidemiological investigation

Basic information on travel and hospitalisation was collected for all patients. The four cases with culture-confirmed ST82 were interviewed using an extended questionnaire focusing on symptoms, risk factors, place of work, daily habits, traffic patterns and recent travel. Home addresses were obtained using the Danish Civil Register and confirmed during the interviews.

Environmental investigation

At the beginning of January 2015, water samples were collected from the homes of three ST82 cases. One case had moved in the meantime and it was not possible to obtain water samples from the previous home. During the incubation period, one case, a professional cleaner, had attended work and water samples were also collected from this location. Two water samples each of 1 L were collected from each of the four addresses. Samples of hot water were collected from the shower hoses at the homes and tap water from the workplace of the cleaner as a first flush sample and an additional one after 20 s of flushing. The owners were instructed not to clean the shower before the visit or use it on the day of the visit. Additionally, a swab was taken from the tap of the shower. Samples were analysed at the National *Legionella* Reference Laboratory at SSI. During the visit, cases were re-interviewed, using an event calendar as a memory aid.

Microbiological investigation

Culture of *Legionella* from water samples

Water samples were analysed in accordance with ISO 11731, which consisted of direct plating (2 x 0.5 mL) as well as plating (0.1 mL) concentrated (x100) sample material after filtration (0.2 µm filter), and (x1,000) after centrifugation. The material was seeded on selective media Modified Wadowsky Yee (MWY) and Glycine-Vancomycin-Polymyxin B sulphate-Cycloheximide (GVPC) agar plates (both from Oxoid, GmbH) and was incubated at 37 °C in a humid atmosphere for 7–8 days. If dense growth of background bacteria was observed, acid (HCl-KCl buffer, pH 2.2 for 5 min) and heat treatment (50 °C for 30 min) on the concentrated samples were applied.

Diagnostic methods at the Department of Clinical Microbiology

LD was diagnosed using a combinatory approach. First, real-time PCR was performed on respiratory samples to detect and differentiate between *Legionella* spp. and *L. pneumophila* [3]. PCR-positive samples were cultured for *Legionella* spp. on both Modified Wadowsky Yee-Oxoid (MWY-O) and Buffered Charcoal Yeast Extract (BCYE) agar plates (in-house media) by standard technique. Colonies identified as *L. pneumophila* with MALDI-TOF (MALDI-TOF) were referred to SSI for typing including serotyping by the Dresden panel (including MAb 3 of the international panel) of monoclonal antibodies to determine the serogroup and subgroup if applicable [4,5]. Urine samples were examined for *L. pneumophila* serogroup 1 soluble antigen (UAg) by the Alere BinaxNOW assay according to instructions from the manufacturer.

Sequence-based typing on clinical isolates and PCR-positive samples

Genomic DNA from the submitted isolates was extracted by the QIAamp DNA Mini kit (Qiagen) and *L. pneumophila* isolates were genotyped using the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Legionella Infections (ESGLI) consensus sequence-based typing (SBT) scheme, which allows assignment of seven ordered alleles to an allelic profile representing a sequence types (ST) [6,7]. The trace files with the obtained sequences were analysed using the Legionella SBT quality tool at the website (http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php) to retrieve STs and ensure the quality of sequences. PCR-positive samples negative for L. pneumophila by culture were subjected to direct nested SBT as previously described [8].

wzm PCR (serogroup 1-specific PCR)

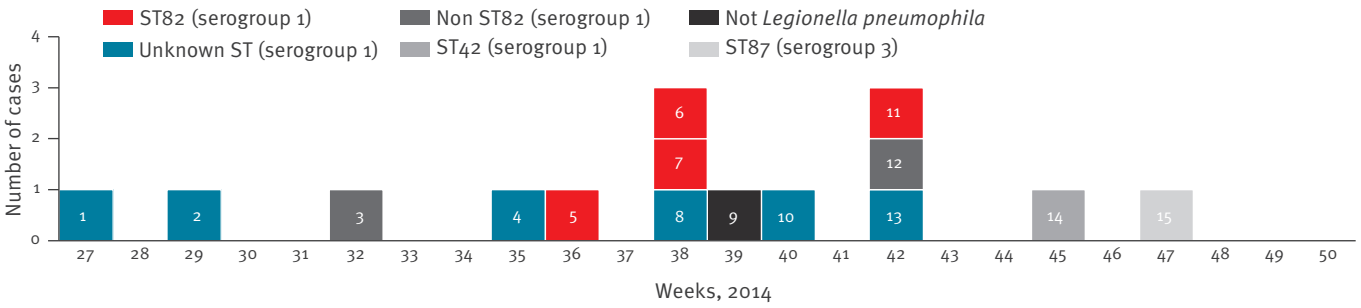
Available PCR-positive culture-negative samples were investigated by a real-time PCR targeting the serogroup 1 marker *wzm* [9,10] to discriminate between *L. pneumophila* serogroup 1 and non-serogroup 1. A positive result in the *wzm* PCR was considered as serogroup 1 whereas PCR-positive sample with a cycle threshold (CT) value of ≤ 35 in the specific *L. pneumophila* real-time PCR (*mip* specific PCR, in-house, SSI) but with no amplification with the *wzm* primers was considered as non-serogroup 1.

Whole genome sequencing

Genomic DNA used for SBT was also used for whole genome sequencing (WGS) using the Illumina MiSeq platform to obtain 251-bp paired-end reads according to the instructions from the manufacturer, or the Illumina HiSeq platform with 100-bp paired-end reads. The isolates of the four ST82 cases were initially analysed together with three other epidemiologically

Figure 1

Cases with Legionnaires' disease diagnosed at the Department of Clinical Microbiology, regional hospital, Denmark, 30 June–19 November 2014 (n = 15)



ST: sequence type.

unrelated community-acquired ST82 isolates from Denmark; two cases from Funen in 2011 and one case from Jutland in 2012. None of the 488 genomes available at ftp.ncbi.nlm.nih.gov/genomes/refseq/bacteria/Legionella_pneumophila were ST82 and eligible for inclusion. However, additional sequences were subsequently included in the analysis - one sequence from a ST82 isolate from 2015, Jutland in Denmark, and four ST82 sequences from the United Kingdom (UK) [11], where one sequence originated from an isolate of a travel-associated case. This resulted in a total set of 12 whole genome sequences for the investigation (Table 1).

Identification of single nt polymorphism (SNP) variants was performed using NASP 1.0 (http://tgennorth.github.io/NASP/) by aligning sequence reads from the 12 *Legionella* isolates against the chromosome of *L. pneumophila* subsp. *pneumophila* str. Lorraine (GenBank accession number: NC_FQ958210) using the Burrows-Wheeler Aligner (BWA) [12] after removal of duplicated regions in the reference using NUCmer [13,14]. The Lorraine strain was chosen as reference as it was the closest closed reference available (as determined by k-mer analysis https://cge.cbs.dtu.dk/services/KmerFinder/); the Lorraine strain is ST47 and shares four of the seven SBT loci with ST82 (*flaA* (allele number 5), *asd* (number 22), *proA* (number 6) and *neuA* (number 6).

Variants were identified using the Genome Analysis Toolkit (GATK) Unified Genotyper, and all SNPs that did not meet a minimum coverage of 10 or that were present in < 90% of the base calls were excluded. High-density regions of SNPs including those derived from recombination events were removed using Genealogies Unbiased By recomBinations In Nt Sequences (Gubbins) v1.4.4 [15] with default settings. Phylogenetic trees were constructed using the maximum-likelihood algorithm implemented in PhyML at http://www.atgc-montpellier.fr/phyml-sms/with Smart Model Selection using the Bayesian Information Criterion with 100 bootstrap replicates. The Illumina sequences generated from the 8 Danish *L. pneumophila* isolates described in this study were submitted to the European Nt Archive (ENA; http://www.ebi.ac.uk/ena), accession numbers listed in Table 1 study ID PRJEB21315. Accessory genomes were analysed by using assembled Genomic reads by Spades v. 3.5.0 [16]. Prokka v. 1.2 [17] was used for gene annotation and Roary v. 3.6.0 [18] was applied to define gene 'presence/absence'. Results were inspected manually.

Results

Microbiology and standard typing

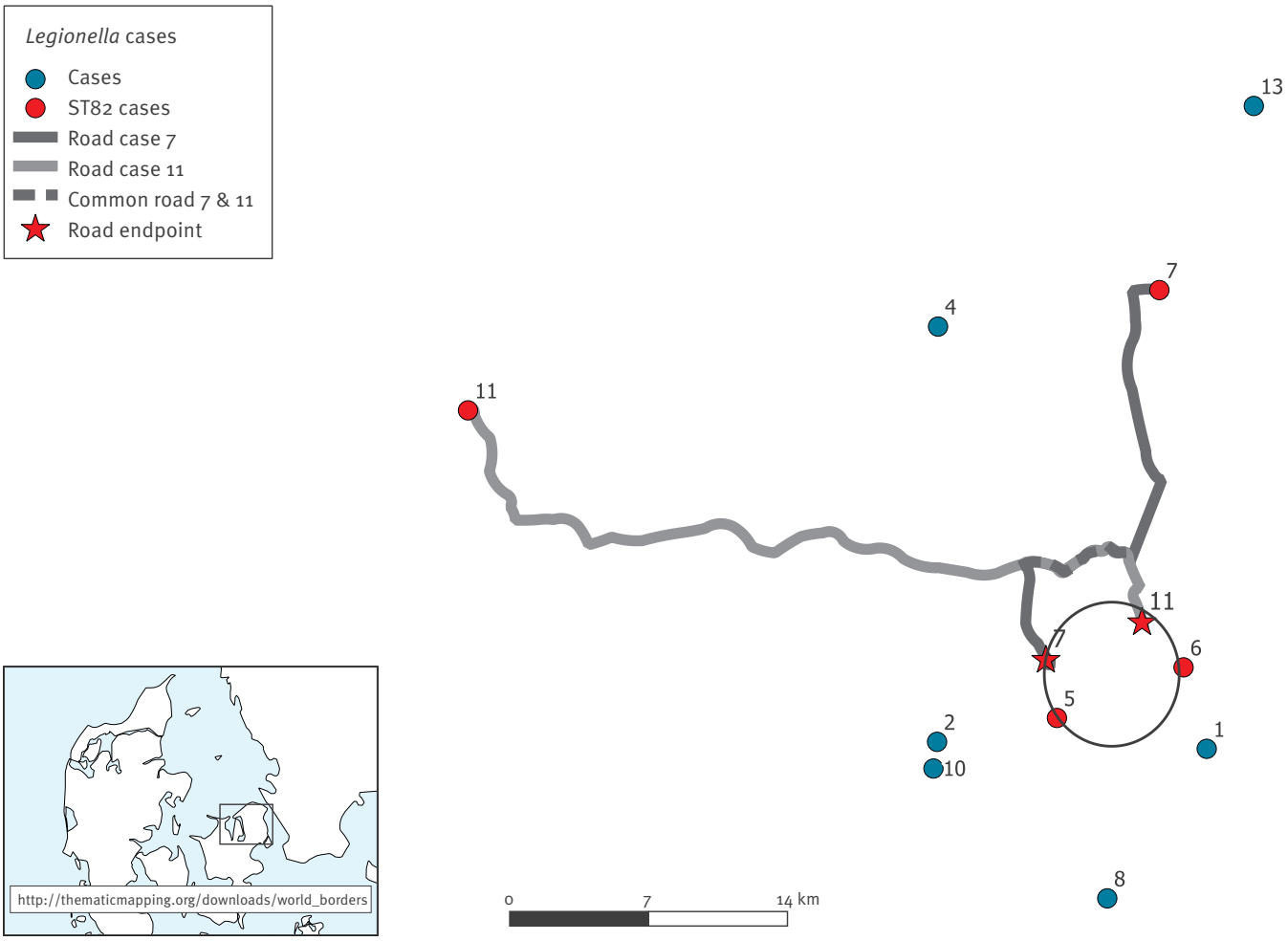
Between 30 June and 19 November 2014, 15 patients fulfilled the outbreak case definition in North Zealand. According to the EU Legionnaires' disease definitions, 13 of the cases were confirmed cases (6 by culture and seven by UAg test) and two were probable cases. Of the 15 cases, the isolates from four patients were typed as *L. pneumophila* serogroup 1, subgroup Allentown/France, ST82, as previously mentioned. These four cases were used to further define the cluster as a putative ST82 outbreak.

Based on subsequent typing data, five of the 15 cases could be excluded from the putative ST82 outbreak as the ST was not ST82. These included two culture-positive cases, one characterised as serogroup 3 ST87, and the other as serogroup 1, subgroup Benidorm, ST42. One culture-negative case was PCR-negative for *L. pneumophila* but positive for *Legionella* spp. (a non-*pneumophila* case). One culture-negative case was PCR-positive for *L. pneumophila* but negative in the *wzm* assay (and nested SBT revealed three of seven alleles confirming it as a non-ST82 case) and another was serogroup 1 (*wzm*-positive) but non-ST82 (nested SBT typing revealed five of seven alleles which suggested a ST42 [data not shown]).

Six cases were left as possible cases for the putative ST82 outbreak, all of which were diagnosed by urinary

Figure 2

Mapping of residence of the cases of Legionnaires' disease, or trajectory during their incubation period, Zealand, Denmark, 30 June–19 November 2014 (n = 10 cases)



The circle on figure indicates a small geographical area of 7 km in diameter, which the four ST82 cases were linked to. Case 7 drove by car from point 7 to the location indicated by a red star shape (during the incubation period). Case 11 drove by car from point 11 to the location also indicated by a red star, leaving a common path of ca 5 km long between case 7 and 11.

antigen (UAg) and two also by PCR (*L. pneumophila*). However, no sample material was stored for *wzm* PCR or nested SBT typing. In conclusion, the putative ST82 outbreak included six possible ST82 cases and four cases with culture-confirmed ST82 infection. In addition, no changes during 2010–2014 were identified in the laboratory procedure or diagnostic tests used for LD at DCM at the regional hospital which could have influenced the detection of LD cases.

Descriptive epidemiology

The mean age of the six possible (marked in dark blue in Figure 1) and four confirmed ST82 cases of the putative ST82 outbreak (marked in red in Figure 1) was 65.7 years, and eight of 10 cases were men. All patients were hospitalised. One of the 15 cases was fatal.

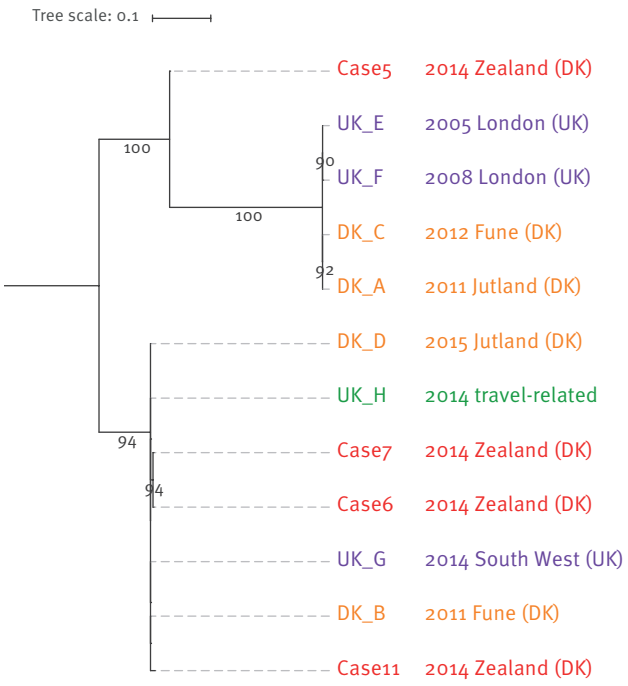
Through the interviews, possible exposures during the incubation period were evaluated. No places of exposures related to shared events, local watermills, cooling towers, major constructions, or irrigation of recreational areas such as sports facilities etc., were in common to the cases, and therefore no additional environmental samples were collected.

The home addresses for all outbreak cases were plotted and major traffic patterns for the ST82 cases were included in the map (Figure 2). Two cases (number 11 and 7) had travelled approximatively 5 km on the same route by car during the incubation period. One case (number 6) mainly stayed in the town which case number 7 sometimes visited. However, there were no confirmed visits during the incubation period. In addition, case number 5 had remained close to home. Combined, this placed all four ST82 cases within a

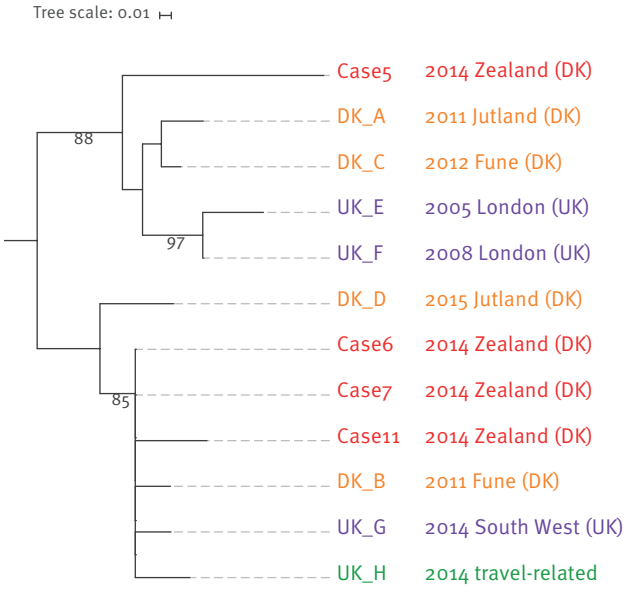
Figure 3

Phylogenetic analysis of the ST82 genomes demonstrates heterogeneity between the suspected outbreak isolates

A. All identified SNPs within the core genome of the ST82 collection



A. Only the non-recombinant core genome SNPs



SNP: single nt polymorphism; DK: Denmark; United Kingdom: UK.

small geographical area of 7 km in diameter ('the ST82 area'). The homes of the six possible cases were more distant, with a maximum of 42 km between case 13 and case 8. While case 8 had visited the southern part of the 'ST82 area' during the incubation period, in relation to work, the remaining five had not travelled to this area. However, cases 1, 2 and 10 lived close to the 'ST82 area', but the homes of cases 1 and 10 were separated by 13 km.

Environmental investigation

Analysis of the water samples revealed that one home was contaminated with L. pneumophila serogroup 1, subgroup Oxford/OLDA, ST1 (24,000 CFU/L in the first flush sample). The water samples from homes of the two additional cases were negative for *Legionella*. The water samples from the workplace were also contaminated with L. pneumophila serogroup 1, subgroup Oxford/ OLDA, ST1 but also with serogroup 4, subgroup Portland (›600,000 CFU/L in the first flush sample). The workplace was notified of the finding, but no information of further sampling is available.

Whole genome sequencing

The phylogenetic analysis (Figure 3), based on an alignment comprising›90% of positions in the reference chromosome, showed that all included isolates could be separated into two main clades (I and II).

Clade I contains two isolates from Denmark obtained in years before the outbreak (DK_A, 2011 and C, 2012) together with two isolates from London, UK (UK_E, 2005 and F, 2008). Case 5's isolate (clade I) is separated from the three other outbreak ST82 isolates located in clade II by ca 1,700 SNPs when putative recombinant regions are respectively included in the SNP analysis (Figure 3A). When such recombinant regions are excluded, this case's isolate sequence differed from the others by 16 to 21 SNPs (Figure 3B).

Shown are rooted maximum likelihood phylogenies which were reconstructed using, A) all identified SNPs within the core genome of the ST82 collection, and B) using only the non-recombinant core genome SNPs. Names listed in red are the investigated outbreak strains of ST82. The scale bar indicates substitutions per site.

Clade II contains three of the ST82 outbreak isolates (case 6, case 7, and case 11) together with unrelated isolates from Denmark (DK_B, 2011 and D, 2012) and the South West region of the UK (UK_G, 2014), as well as an isolate obtained in the UK but associated with travel (UK_H, 2014). The isolates from cases 6 and 7 were identical, whereas case 11 differed by 118 and four SNPs (relative to isolates from cases 6 and 7) when recombinant regions were included and excluded, respectively. The two main clades (excluding case 5) were separated by ca 3,600 SNPs when recombination regions were included but only by 12 to 21 SNPs when these were removed. Thus, the genetic variation

Figure 4

Predicted recombined regions in isolates of the ST82 lineage included in the investigation of a putative outbreak in 2014 conducted in Denmark

DK: Denmark; United Kingdom; UK.

The reference is *Legionella pneumophila* subsp. *pneumophila* str. Lorraine, GenBank accession number NC_FQ958210.

A phylogenetic tree constructed using single nt polymorphisms (SNP)s that remain after the identification and removal of recombined regions by Gubbins algorithm is shown. The recombined regions are indicated by blue blocks, which are unique to a single isolate, and red blocks, which are shared by multiple isolates through common descent. The horizontal position of the blocks represents their position in the core genome alignment.

within the two clades due to de novo mutation was very limited.

In clade I, all four strains shared the same putative regions of recombination (Figure 4); thus the intra-clade SNP distances were similar irrespective of whether these regions were included or excluded (3 to 9 SNPs). The isolate from case 5 shares recombinant regions with isolates in clade I (Figure 4), and clusters together with these isolates when recombinant regions are removed (Figure 3B); this is a strong indication of a common evolution for all the isolates in clade I and for the isolate from case 5.

The observed variation within clade II was higher than within clade I with zero to 118 SNPs between isolates, compared with zero to 10 with or without recombinations, respectively. The isolates from outbreak cases 6, 7 and 11 display signals of recombination that were not shared by any of the remaining four isolates (DK_B, DK_D, UK_G and UK_H) in this clade (Figure 4). The identical isolates from cases 6 and 7 were phylogenetically more related to UK_G, UK_H and DK_B with only 43 to 44 SNP and two to three SNP differences with and excluding identified regions of recombination, than to the last suspected outbreak isolate of case 11 with 118 and four SNPs, respectively (Figure 3).

The maximum time span between the isolation dates of any two isolates in the same clade was four and a half years between DK_B and DK_D. No recombination events distinguishing these two isolates were identified in the analysis, and seven SNPs separated the isolates. In the analysis of the accessory genome, 540 genes were identified among the 12 isolates, however these contained no known pathogenic virulence genes.

Table

Isolates that were analysed using whole genome sequencing (n = 12)

Case ID	Identifier[a]	Year	Country	Region	Acquired	Sample accession	Experiment accession
Case 5	EULV9728	2014	DK	Zealand	CA	ERR2009177	ERX2068934
Case 6	EULV9736	2014	DK	Zealand	CA	ERR2009176	ERX2068935
Case 7	EULV9737	2014	DK	Zealand	CA	ERR2009171	ERX2068936
Case 11	EULV9735	2014	DK	Zealand	CA	ERR2009170	ERX2068933
DK_A	EULV9728	2011	DK	Jutland	CA	ERR2009172	ERX2068937
DK_B	EULV10974	2011	DK	Funen	CA	ERR2009173	ERX2068938
DK_C	EULV10973	2012	DK	Funen	CA	ERR2009174	ERX2068939
DK_D	EULV10972	2015	DK	Jutland	CA	ERR2009175	ERX2068940
UK_E [b]	EULV00167	2005	UK	London	UNK	NA	
UK_F [b]	EULV3067	2008	UK	London	CA		
UK_G [b]	EULV10052	2014	UK	South West	CA		
UK_H [b]	EULV10407	2014	UK	UK	TA		
Reference	Lorraine NC_FQ958210[c]						

DK: Denmark; CA: community-acquired; United Kingdom: UK; UNK: Unknown; TA: travel-associated.

[a] Sequence-based typing (SBT) data for all isolates were submitted to the European Society of Clinical Microbiology and Infectious Diseases Study Group for Legionella Infection (ESGLI)'s Database for *Legionella pneumophila* (http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php).

[b] Sequence published in Mentasti et al. 2017 [11]

[c] The GenBank accession number of the reference.

The analysis of the accessory gene content confirmed the relatedness between isolates from case 6 and 7 (data not shown).

Discussion

In this study, we present the results of the epidemiological, environmental and genetic (typing) investigations of a small putative ST82 LD outbreak with four ST82 cases and six possible cases (one fatal) that occurred between June and November 2014 in Northern Zealand, Denmark.

Four cases of this suspected outbreak were caused by ST82.This ST is not common in Denmark and, as mentioned above, has not been seen in this geographical area before or after summer/autumn 2014. According to the ESGLI *L. pneumophila* SBT database, ST82 is not a very common ST in Europe or elsewhere with only 125 of 10,929 submitted isolates (as at 17 August 2016). All submissions are from Europe and mostly from France (n = 78) followed by UK (n = 12) and the Netherlands (n = 10). Only four of the isolates in the database are of environmental origin, however, none was obtained for this study. This distribution resembles that of the more common and closely related ST47 (which includes the Lorraine reference strain), with 612 entries of which only 10 are environmental. This type is the most common ST among clinical isolates in the Netherlands, England/Wales and Belgium [19] but many of the submissions are from France (n = 265).

The four ST82 cases in the putative outbreak clustered in regards to sero-/subgroup and ST (ST82), but, importantly, also in time and geographic region of residence and commuting. However, no common place(s) of exposure could be determined. We found *L. pneumophila* in water samples from sites where two patients had been during the incubation period, but none of the isolates were ST82. The lack of ST82 in the water samples (homes and workplace) could imply one or more external sources. The epidemiological data placed all four ST82 cases within a small geographical area of 7 km in diameter, thus the focus of the investigation was on these cases. Two of them had a common driving itinerary, along a route affected by road construction works which caused long queuing times, so it was speculated that cooling towers along their route might be the source of the outbreak. Both cooling towers and aerosolised water from industrial settings have previously been implicated in LD outbreaks [20–24]. However, in contrast to other European countries, Denmark does not have a cooling tower register, which limits the investigation and the environmental sampling from these sources [25]. No environmental ST82 isolates were obtained from any putative sources and no environmental ST82 isolates were available to be included in the analysis.

WGS was applied to obtain further clarification into the origin and relatedness of the four cases. It has recently been described that *L. pneumophila* can be rather genetically heterogeneous even among isolates within defined outbreaks [26,27]. However, a recent study of 10 separate *L. pneumophila* serogroup 1 'outbreaks' in New York State [28] showed that isolates from almost all outbreaks formed outbreak specific clusters without any overlap, and isolates within these clusters differed by < 5 SNPs in most instances. In

this investigation, a variety of isolates from unrelated cases of LD that occurred in both Denmark and the UK were included under the assumption that isolates from the same source would show less variation compared with epidemiologically unrelated isolates. However, our WGS-based phylogenetic analysis, performed before and after the removal of recombined regions, challenges the hypothesis of a common source for the four investigated ST82 outbreak isolates. The isolate from case 5 differs from the other three ST82 isolates by ca 1,700 SNP differences and at least six recombined regions. Secondly, cases 6 and 7 were identical based on our analyses, but more closely related to (i) a travel-associated isolate obtained from the UK, (ii) the UK isolate from 2014, and (iii) an epidemiologically-unrelated Danish isolate from 2011, than to the isolate from case 11. In addition, case 11 differs from cases 6 and 7 by three putative recombination events. Other *L. pneumophila* cases have been described as part of an outbreak based on strong epidemiological links with 15–17 SNPs between isolates [29,30].

As recombination has been shown to be a significant driving factor in *Legionella* evolution [31], it is important to consider this process when inferring relatedness based on core diversity. Our data indicate that the diversity within the ST82 clade is very limited when disregarding the effect of recombination and highlight the importance of including unrelated isolates of the same ST in the WGS analysis when investigating an outbreak in genetically highly similar clones. Around 99% of the SNP differences that distinguished the case 5 isolate from isolates from case 6, 7 and 11 were found in the recombinant regions. The most recent common ancestor of the isolate from case 5 and the isolates from 6, 7 and 11 must have existed before 2005 (9 years prior) as the oldest isolate in clade I is from 2005. Other publications also highlight the importance of evaluating and including observations of recombination in outbreak investigations [26,31]. McAdam and colleagues described one patient with two genetic subtypes which differed by 20 core genome SNPs (after removal of recombination events) during a cluster detection in Edinburgh and concluded, based on the short timescale between the exposure and isolation, that multiple subtypes must have co-existed in the source before acquisition [27]. Coscollá and colleagues also described mixed infections with different subtypes of *L. pneumophila* in outbreak patients [32]. Hence, the observed difference between the isolate from case 11 and two isolates from cases 6 and 7, respectively, is comparable to their findings and does not alone exclude a common source for the three cases. However, the results obtained by the inclusion of the epidemiologically unrelated isolates indicate that the cases could be unrelated (i.e. from different sources) despite their close genetic cluster.

Our finding that epidemiologically unrelated isolates sampled many years apart can differ by as few as two SNPs implies a very low evolutionary rate by point mutation for *L. pneumophila* (<1 SNP/genome/year) and perhaps the existence of a dormancy stage within the life cycle. Underwood et al. also found that some isolates of the same ST (interestingly the close ST47) that were separated by several years and geographic location differed by just four SNPs [31]. The diversity could be different in other *L. pneumophila* lineages, however similar results have recently been shown in several other STs 1, 23, 36, 37, 47 and 62 [33,34].

WGS analysis has emerged as the new and highly discriminatory tool for microbial genotyping. Obtaining data, analysing and understanding the outputs can be challenging both in regards to timely analysis of data and due to the lack of standardisation, which makes rapid sharing of these cumbersome. Recent work, however, attempts to standardise the typing of *Legionella* [35]. The national surveillance in Denmark is still based on ST typing with subsequent WGS on selected clusters. Real time analysis by WGS may have ended the suspicion of a larger outbreak very early in the process as the isolate of case 5, the chronologically first ST82 case, was clearly different from the following two ST82 isolates, which on the other hand were indistinguishable. ST82 has not been detected in any cases in this region of Denmark either before or after this period in 2014. Therefore, the situation with four cases with the same rare ST diagnosed within a few weeks was extraordinary.

The six possible cases were not included in the WGS analysis, as the primary diagnoses were performed using UAg and no isolates were available. An attempt to culture the causative agent in all cases of LD is important, but even without isolation, the examination of positive respiratory samples is of great value to include or exclude cases from an outbreak as shown recently by Mentasti and co-workers [10] and in this study. Despite the fact that isolate submission is only voluntary in Denmark, the continued submission of isolates is of pronounced value for national surveillance, as well as submission of positive PCR samples where isolation is not possible.

We conclude that our data contribute to the discussion on how *L. pneumophila* outbreaks should be interpreted using WGS data and contribute to the general knowledge about the diversity within *L. pneumophila*. In this investigation, the microbiological results do not directly point to a single source outbreak and we were left without a clear epidemiological link. This highlights the importance of timely interviews of cases to explore all possible exposures. However, the presented data also show the importance of including epidemiologically and spatially unrelated isolates into WGS-based analysis as well detecting and evaluating the effect of recombination on the interpretation.

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Conflict of interest

None declared

Authors' contributions

SS: Participated in the outbreak investigation, data analysis and drafted the manuscript. MS: Analysed the WGS and Gubbin data and contributed to the writing of the manuscript. CK: Participated in the outbreak investigation and writing of the manuscript. BL: Analysed the WGS, Gubbin and accessory genome analysis. JMB: Analysed the patient samples and contributed to the writing of the manuscript. RFP: Analysed the DNA extractions arriving at SSI. SD: Analysed the WGS data and contributed to the writing of the manuscript. SAU: Identification and characterisation of Legionella isolates, has participated in the outbreak investigation and the writing of the manuscript.

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Research article

Baseline incidence of intussusception in early childhood before rotavirus vaccine introduction, the Netherlands, January 2008 to December 2012

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Intussusception is a rare, potentially life-threatening condition in early childhood. It gained attention due to an unexpected association with the first rotavirus vaccine, RotaShield, which was subsequently withdrawn from the market. Across Europe, broad variations in intussusception incidence rates have been reported. This study provides a first estimate of intussusception incidence in young children in the Netherlands from 1 January 2008 to 31 December 2012, which could be used for future rotavirus safety monitoring. Our estimates are based on two different sources: electronic medical records from the primary healthcare database (IPCI), as well as administrative data from the Dutch hospital register (LBZ). The results from our study indicate a low rate of intussusception. Overall incidence rate in children < 36 months of age was 21.2 per 100,000 person-years (95% confidence interval (CI): 12.5–34.3) based on primary healthcare data and 22.6 per 100,000 person-years (95% CI: 20.9–24.4) based on hospital administrative data. The estimates suggest the upper and lower bound of the expected number of cases.

Introduction

Rotavirus infections are a leading cause of severe diarrhoeal illness in infants and young children [1]. As demonstrated by a number of studies, rotavirus vaccines are effective in preventing severe diarrhoeal illness caused by certain rotavirus serotypes [2,3]. In 1999 however, the first rotavirus vaccine, RotaShield (Wyeth Laboratories, Inc., Pennsylvania, United States), was voluntarily withdrawn from the market due to an unexpected association with intussusception [4]. Intussusception is a serious condition that can be described as the invagination of a proximal segment of the bowel into the distal bowel. If left untreated, the blood flow can become compromised, leading to bowel infarction and perforation. In 2006, two second generation rotavirus vaccines, Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq (MSD vaccines, Lyon, France), were approved for marketing in Europe. In 2009, the World Health Organization Strategic Advisory Group of Experts (SAGE) recommended the use of rotavirus vaccines in all national immunisation programs, and by 2016, 11 countries of the European Union had included rotavirus vaccination in their national vaccination programme [5]. Although large-scale pre-licensure clinical trials did not identify an increased risk for intussusception, post-licensure data suggested a small increase in risk of intussusception that was closely linked to the age of vaccination after rotavirus vaccination with both licensed vaccines [6-11].

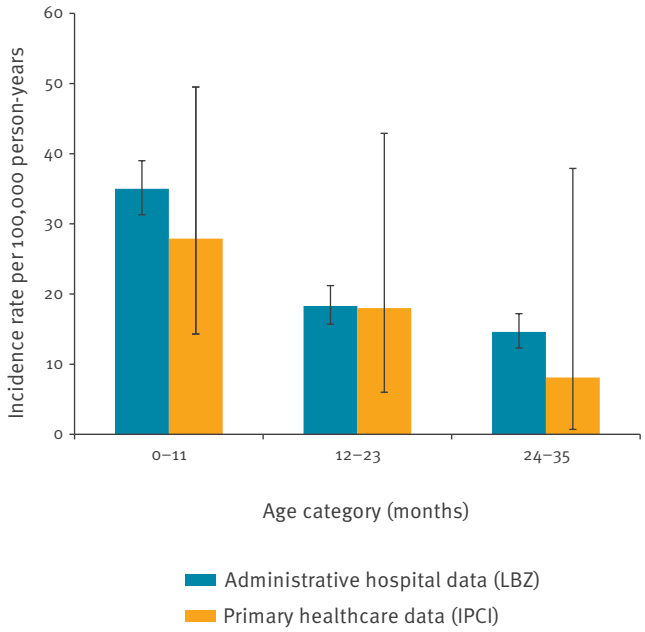
The aetiology of primary intussusception in young children remains unclear. Intussusception is most common between 5 and 7 months of age [12]. Approximately 60% to 75% of children diagnosed with intussusception are younger than 1 year of age, and approximately 80% to 90% are younger than 2 years of age. Most episodes occur in otherwise healthy children with a male to female predominance of ca 3:2 [13].

Ultrasonography is the method of choice to detect intussusception. Ultrasound-guided reduction using hydrostatic or pneumatic pressure by enema is the treatment of choice. Surgical treatment is indicated when ultrasound-guided reduction is incomplete or in case perforation is suspected.

Sentiments towards the importance of vaccination in general are positive overall but confidence in vaccine

Figure 1

Intussusception incidence rate in children < 36 months of age per 100,000 person-years by age and data source, the Netherlands, 1 January 2008–31 December 2012

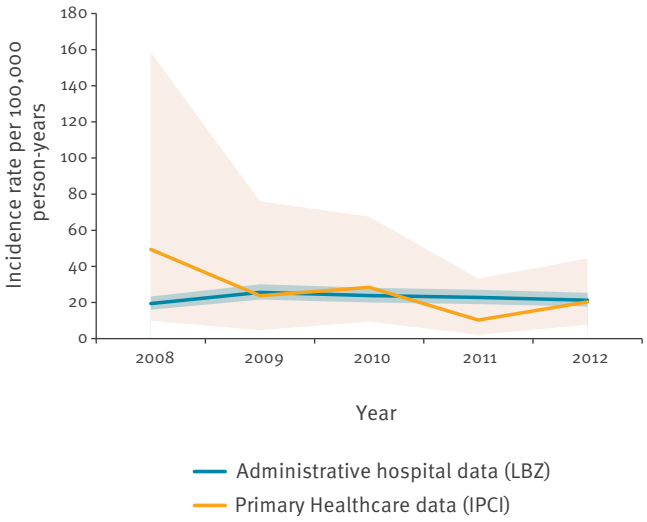


IPCI: Integrated Primary Care Information database; LBZ: Landelijke Basisregistratie Ziekenhuiszorg database.

Vertical lines represent the 95% confidence intervals.

Figure 2

Intussusception incidence rate in children < 36 months of age per 100,000 person-years by year and data source, the Netherlands, 1 January 2008–31 December 2012



IPCI: Integrated Primary Care Information database; LBZ: Landelijke Basisregistratie Ziekenhuiszorg database.

Shaded areas represent the 95% confidence intervals.

safety is less positive, particularly in the European region [14]. In the Netherlands, where the impact of rotavirus vaccine is considered modest [15], concerns about vaccine safety may lead to vaccine hesitancy and decreased vaccination coverage for vaccine-preventable diseases in general [16]. Knowledge of the background incidence rates of possible adverse events is a crucial part of assessing possible vaccine safety concerns. It allows for a rapid observed vs expected analysis and helps to distinguish legitimate safety concerns from events that are temporally associated with but not necessarily caused by vaccination [17]. In the case of rotavirus vaccination, it is important to know the background incidence of intussusception. Studies in Europe have reported incidence rates of intussusception between 24.2 and 60.4 per 100,000 person-years [18-23] and show a decline over time [19,22]. Methods used to estimate these incidence rates differ in age of source population, length of study period, and detection and validation of cases.

To date, the use of rotavirus vaccines in the Dutch population can be considered negligible. Rotavirus vaccines are not included in the Dutch national vaccination programme, are not recommended for routine use and are not reimbursed by the health insurance. To support future rotavirus vaccine safety surveillance in the event that rotavirus vaccine would be introduced in the Dutch national vaccination programme, this study aims to calculate the baseline incidence rates of intussusception in the Netherlands.

Methods

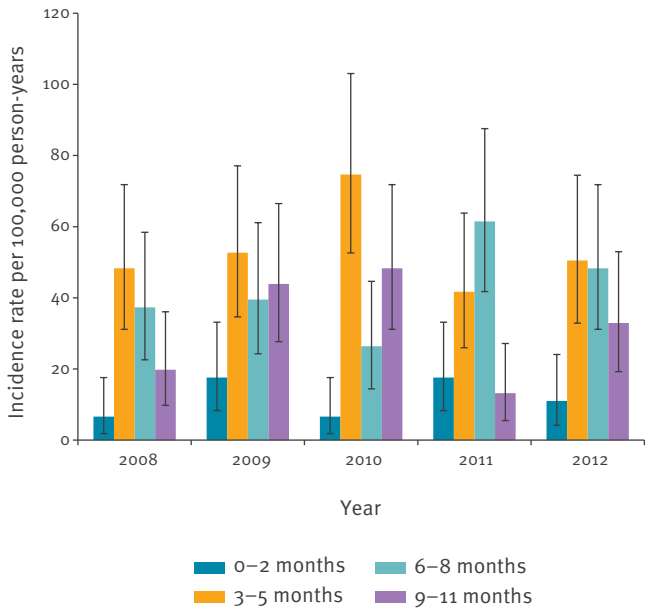
Data sources

For this study two different data sources that capture a partially overlapping source population were used: an administrative hospital discharge database and a primary healthcare database. Administrative hospital discharge data were obtained from the Dutch hospital register, Landelijke Basisregistratie Ziekenhuiszorg (LBZ). Hospitals and university medical centres in the Netherlands have a legal and statutory obligation to collect and provide electronic administrative data to the LBZ database on a monthly basis. The LBZ database covers more than 80% of the total Dutch population of 17 million people. It contains anonymised data on hospital admissions, outpatient consultation and emergency department visits including medical diagnoses, as well as patient-specific data such as age and sex [24]. Coding of discharge diagnoses is performed by participating hospitals according to the International Classification of Diseases, Ninth Revision (ICD-9) [25]. A validation study showed high accuracy of coding and concluded that the discharge data are generally of high quality [26].

The second source was a primary healthcare database, the Integrated Primary Care Information (IPCI) database. IPCI is a longitudinal observational database

Figure 3

Intussusception incidence rate in children < 12 months of age per 100,000 person-years by age category and year based on non-validated cases from the LBZ database, the Netherlands, 1 January 2008–31 December 2012



LBZ: Landelijke Basisregistratie Ziekenhuiszorg.

The LBZ database contains administrative hospital data.

Vertical lines represent the 95% confidence intervals.

created specifically for pharmaco-epidemiological studies [27]. It contains anonymised data, including notes from computer-based medical records of around 600 general practitioners (GPs) located in the Netherlands. IPCI contains information on more than 1.1 million patients from over 200 participating GP practices. The age and sex distribution of the population is representative for the Netherlands. In the Dutch healthcare system, the GP acts as a gatekeeper for all medical care. It is estimated that more than 75% of the Dutch population in the age group 0–3 years will visit their GP at least once per year [28]. In the event intussusception is suspected, the patient will be referred to hospital for confirmation and treatment. Following consultation at the hospital, it is standard practice to forward the details of the consultation and the outcome to the GP. In the rare event a patient by-passes the GP, the hospital will also forward the details of the consultation and the outcome to the patient's GP. Therefore, patients' medical records at GP practices are likely to contain all relevant medical information.

Study design and population

Cases were retrieved differently from the two data sources. From the LBZ hospital discharge database, all cases with a primary or secondary discharge diagnosis of intussusception (ICD-9 CM code 560.0) in children aged between 0 and 36 months (i.e. children aged 0–35 months) at admission were retrieved for the period from 1 January 2008 to 31 December 2012. Cases with a secondary diagnosis of intussusception were reviewed by a paediatrician to determine whether the combined set of ICD codes for the admission was compatible with a new occurrence of intussusception, taking comorbidities, patient age and primary discharge diagnosis into account. Possible duplicate reports because of patient transfers were identified based on sex, birthdate and date of diagnosis, and were excluded.

Table 1

Incidence rate of intussusception per 100,000 person-years based on validated cases from the Integrated Primary Care Information (IPCI) database, the Netherlands, January 2003–December 2012

Year	Cases (n)	Person time (person-years)	Incidence rate per 100,000 person-years	95% CI
2003	0	2,226	NA	NA
2004	1	2,323	43.0	3.9–200.7
2005	0	4,549	NA	NA
2006	0	978	NA	NA
2007	0	1,495	NA	NA
2008	2	4,045	49.4	9.9–158.5
2009	2	8,435	23.7	4.7–76.0
2010	4	14,087	28.4	9.5–67.5
2011	2	19,326	10.3	2.1–33.2
2012	5	24,649	20.3	7.7–44.5

Based on the data captured by IPCI, we constructed a dynamic cohort. Initially, we attempted to assess the incidence rate over a period of 10 years, from 1 January 2003 to 31 December 2012. However, the addition of many new practices to the IPCI database in 2007 caused the observation time to vary considerably over the course of the 10-year study period and we subsequently restricted the analysis to a period of 5 years, from 1 January 2008 to 31 December 2012. This yielded a more stable population (Table 1).

We only selected children who were born during the study period and who had contributed longitudinal data to the IPCI database from birth onwards. Follow-up started from birth and continued until the date the patient became a case, the patient reached the age of 36 months, the patient died, the patient was transferred out of the GP's practice, the date of last data collection from the general practice or the end of the study period was reached, whichever date came first. In the IPCI database, cases were identified by automated scanning of keywords in GPs' notes in the medical records. The complete medical records of all potential cases were reviewed by a medical doctor for details alluding to additional diagnostic procedures or received treatment. An identified case was considered a true case if the medical journal of that particular patient contained results from ultrasound examination

TABLE 2

Study cohort details for investigation of intussusception incidence rates using the IPCI database, the Netherlands, 1 January 2008–31 December 2012

Study cohort	Time period	
	1 Jan 2003–31 Dec 2012	1 Jan 2008–31 Dec 2012
Study population (n)	155,880	144,617
Person time of follow up (person-years)	82,113	70,542
Number of intussusception cases (n)	16	15

IPCI: Integrated Primary Care Information.
The IPCI database contains general practitioner medical records.

confirming the diagnosis and/or details regarding receiving treatment specific for intussusception such hydrostatic reduction. Cases with an actual diagnosis of intussusception were subsequently classified by level of evidence using the web-based Automated Brighton Collaboration Case definition tool (ABC-tool) for intussusception. Level one corresponds to the highest level of diagnostic evidence and level three corresponds to the lowest [29]. We subsequently compared LBZ and IPCI data for those years where comparable data were available.

Analysis

Age-specific incidence rates were calculated from data in each of the two data sources using the number of intussusception cases from that source as a numerator and the study population as denominator. Since dominator data are not available in the LBZ database, approximate denominators based on national population data from Statistics Netherlands (CBS) were used, assuming that 80% of the population would be covered in the LBZ database [30]. In the IPCI database, the underlying study cohort could be well defined in terms of the number of person-years of follow-up of patients. Incidence rates of intussusception were calculated by dividing the number of incident intussusception cases by the total number of person-years. Incidence rates were calculated by calendar year, age category and sex. Confidence intervals (95% CI) for each estimate were based on the Poisson distribution.

Results

Landelijke Basisregistratie Ziekenhuiszorg (LBZ) database

In the LBZ database, 705 potential cases of intussusception were identified during the study period and 166 duplicate cases were excluded. Based on the remaining 539 identified cases of intussusception, the overall crude incidence rate over the study period from 1 January 2008 to 31 December 2012 was 22.6 per 100,000 person-years (95% confidence interval (CI): 20.9–24.4). Figure 1 shows the age-specific incidence rates; the highest rate was observed in children < 12 months of age.

The incidence rate remained constant over time (Figure 2).

The incidence rate in children < 12 months of age varies considerably by age, but as is expected, the data show that is it more common after the age of three months (Figure 3).

Integrated Primary Care Information (IPCI) database

From the IPCI database, 155,880 children were included in the initial study cohort (Table 2). Within this population, 131 potential cases were detected after a sensitive search for indicators of intussusception in narratives. We subsequently restricted age-specific analysis to the period from 1 January 2008 to 31 December 2012, which yielded a more stable population. In the period from 1 January 2008 to 31 December 2012, the population comprised of 144,617 children. Following manual validation by a medical doctor, 15 cases (14 definite, 1 possible) were classified as incident intussusception (Table 1). When using the ABC-tool, all cases were classified as having insufficient information to meet the Brighton Collaboration case definition of intussusception. This was because none of the cases included any information regarding one of the exclusion criteria: absence of surgical evidence for an alternative diagnosis. Based on the number of cases validated by a medical doctor, the crude incidence during the period 1 January 2008 to 31 December 2012 was 21.2 per 100,000 person-years (95% CI: 12.5–34.3). The incidence was higher in boys than in girls, and was highest in the lowest age category, subsequently decreasing with age (Table 3). Results per age category are provided in Figure 1, and overall incidence per calendar year in Figure 2.

Discussion

This study showed that background incidence rates of intussusception can be estimated using routinely collected healthcare data. The intussusception incidence rate in children < 12 months of age is 27.9 per 100,000 person-years (95% CI: 14.3–49.5) based on cases from the primary healthcare data that were validated, and 35.0 per 100,000 person-years (95% CI: 31.3–39.0) based on the non-validated hospital data.

Table 3

Intussusception incidence rates in children < 36 months of age per 100,000 person-years based on validated cases from the IPCI database, the Netherlands, January 2003–December 2012

Sex and age	Time period			
	1 Jan 2003–31 Dec 2012		1 Jan 2008–31 Dec 2012	
	Incidence rate per 100,000 person-years	95% CI	Incidence rate per 100,000 person-years	95% CI
Overall	20.2	12–32	21.3	12.5–34.3
Sex				
Male	26.7	14.2–46.2	27.7	14.2–49.2
Female	12.7	4.8–27.9	14.6	5.5–32
Age				
0–11 months	24.6	12.6–43.7	27.9	14.3–49.5
12–23 months	19.8	7.5–43.3	18	6.0–42.9
24–35 months	6.9	0.6–32	8.1	0.7–37.9

IPCI: Integrated Primary Care Information.
The IPCI database contains general practitioner medical records.

These estimates are on the lower end of published incidences across Europe. Consistent with previous research [19,22,23,31], the incidence rate in boys was higher than in girls, and was highest in the youngest age group.

When comparing the intussusception incidence rates derived from the IPCI primary healthcare database with those derived from the LBZ hospital database, the results from the IPCI database are validated but less precise, and possibly an underestimation. From the hospital database, we were able to derive precise incidence rates for smaller age categories. However, the coding could not be validated and the incidence rate may possibly be an overestimation. A declining trend over time was not evident. The true intussusception rate is likely to be in between the estimates derived from the primary healthcare database and the hospital database. The advantage of this dual approach is that interpreting the occurrence of future cases after vaccination may be done with or without validation, and we have provided an assessment of the impact thereof.

The advantage of using a primary healthcare, GP-based database such as the IPCI was that case detection did not depend on the validity of coding as free text keyword searches, rather than codes, were used. The availability of medical notes in free text provided a rich source of information, enabling case ascertainment. Although it would be interesting to know the composition of the participating practices, we were not able to identify any evidence that would suggest that the composition of the GP practice is a risk factor for intussusception. Therefore, we consider it unlikely that our estimate is biased by the composition of the GP practices. As a denominator, we were able to use accrued time since birth. However, intussusception is a condition typically diagnosed in a hospital setting. Although it is considered standard practice to forward all relevant hospital patient data to the GP, it cannot be ruled out that some hospital diagnoses were not communicated to GPs or were substantially delayed in terms of being reported back. Since the number of ascertained cases was small, we could only calculate the incidence for age categories of one year and the confidence intervals are rather large.

The majority of first-time rotavirus infections usually occur in infancy. In high income countries, 65% occurs in infants<1 year of age [32]. Therefore, it is recommended that rotavirus vaccination be administered before the age of 6 months. However, the reported incidence of intussusception varies substantially by age during the first 6 months of life [23,33]. Age-specific incidence rates in months or even weeks of age would be very useful in terms of informing rotavirus vaccine safety policy; however, this would require a much larger cohort.

Because the administrative hospital database covers a larger population than the primary healthcare one, we were able to derive more precise incidence rates and incidence rates for smaller age categories. Incidence rates for small age categories is particular valuable information in the context of rotavirus vaccine safety surveillance since studies suggest that the risk of intussusception caused by rotavirus vaccine is primarily in the first week after the first dose, administered in early childhood [6-11]. However, denominator data are not readily available and approximate denominators from population census data have to be used. In addition, case detection in this particular hospital database depends on the accuracy of coding and could not be validated. Published positive predictive values of ICD-9 codes for intussusception range from 75% to 81%, and are even lower when including outpatient department data [34-36]. Therefore, the hospital database estimates may be an overestimation of the true

incidence. Moreover, in contrast to the primary healthcare database, the intussusception cases derived from the hospital database may contain cases of transient intussusception, recurrent intussusception and/or suspected intussusception. In order to further investigate the quality of the estimates derived from the LBZ database, a validation study could be considered. In the future, if hospital data are to be used for intussusception surveillance, distinguishing suspected intussusception cases and transient intussusception cases from true intussusception cases might be of added value.

Conflict of interest

The views expressed in this article are the personal views of the authors and may not be understood or quoted as being made on behalf of, or reflecting the position of, the Dutch Medicine Evaluation Board.

All authors confirm that there are no known conflicts of interest associated with this publication, and that there has been no financial support for this work that could have influenced its outcome.

Authors' contributions

KG acquired the IPCI data, conducted the analysis, interpreted the study findings and drafted the manuscript. JK and PBV acquired the LBZ data, conducted part of the analysis, contributed to the interpretation of the study's findings and provided revisions to the manuscript. SS and DW provided study supervision and input to the manuscript. HM and MS provided study supervision, contributed to the interpretation of the study's findings and provided critical revisions to the manuscript.

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